THE PASSIVE CELLULAR TRANSFER OF DELAYED TYPE HYPERSENSITIVITY TO INTRADERMAL PROCAINE*

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Delayed, tuberculin-type reactions to intradermal procaine can be elicited in patients who develop local allergic edema of the subcutaneous tissues twelve to twenty-four hours following the injection of procaine for regional anesthesia. This type of reaction to procaine or chemically related local anesthetic agents was first described by Adler and Simon in 1949 (1), and its allergic nature was discussed in later reports by Mitchell (2) and by Siegal (3). Clinically, local allergic edema due to procaine is seen most frequently in dental practice where the affected patient develops massive swelling of the soft tissues of the face and cheek on the day following infiltration of the gums with procaine. Similar local reactions are seen after the injection of procaine for minor surgical procedures in any part of the body, or following the injection of procaine penicillin.

The intradermal skin test response to procaine in these patients has all the gross characteristics of the tuberculin reaction: it first appears about four to five hours after the injection of antigen, reaches its maximum intensity in twelve to thirty-six hours, consists of an area of induration surrounded by erythema which at times is pruritic and eczematous in appearance, and may in extremely sensitive cases proceed to central necrosis and sloughing. Of the patients with delayed type intradermal reactions to procaine, only forty per cent have positive patch tests to procaine (4). The remainder have positive intradermal tests but negative patch tests.

Delayed type cutaneous hypersensitivity to procaine is not clinically related to anaphylactic procaine allergy, nor can circulating antibodies to procaine be demonstrated in these cases (2, 3).

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The delayed type of intradermal reaction merely reflects a capacity of the skin and subcutaneous tissues to become edematous in the presence of procaine. In the absence of demonstrable circulating antibodies, it can reasonably be assumed that the antibodies or antibody-like substances responsible for delayed local allergic tissue edema to procaine are cellular in type.

Landsteiner and Chase in 1942 (5) were the first to develop a simple technic for the passive cellular transfer of delayed type hypersensitivity in animals. In man, Lawrence (6, 7) showed that if living leukocytes or extracts of leukocytes isolated from the peripheral blood of donors sensitive to tuberculin were injected intradermally into tuberculin negative recipients, the recipients became temporarily tuberculin-positive. In his experiments, the hypersensitivity of the recipient to tuberculin was not restricted to the site of transfer but could be demonstrated over the entire body surface for a period of several weeks to several months. Further reports by Lawrence and others have shown that delayed type hypersensitivity to additional microbial antigens such as streptococcal M-substance (8), diphtheria toxin (9), cat scratch antigen (10) and coccidiodin (11) can be transferred in the same fashion.

Delayed type hypersensitivity to microbial antigens as detected by intradermal testing has thus been passively transferred in humans by several investigators using different methods and different antigens. On the other hand, attempts to transfer delayed type hypersensitivity to simple chemical substances in humans have, in general, produced much less consistent results. Urbach, in 1925 (12) reported the first successful passive transfer of contact allergy to simple chemical substances. By transferring fluid from the cutaneous blisters of patients suffering from allergic dermatoses, he was able to induce delayed type hypersensitivity to the offending substance at the transfer sites in normal recipients.

If no naturally occurring blisters were present, they were intentionally raised by the application of cantharides plasters. In subsequent investigations, some workers were able to confirm his results, whereas others were unable to obtain successful transfers using the same technic (13).

Prior to 1957, attempts to transfer delayed type hypersensitivity to simple chemical substances with peripheral blood leukocytes rather than blister fluid produced equally inconclusive results (14, 15, 16). However, Epstein and Kligman in 1957 (17) reported consistently successful transfers of delayed type contact hypersensitivity to pentadecyl catechol, 2,4-dinitrochlorobenzene, and paranitrosodimethyl aniline. Recipient hypersensitivity was induced with viable leukocytes, with untreated blister fluid, with cell free blister fluid or with whole blood transfusions. Good (18) in the same year reported the successful transfer of delayed type contact hypersensitivity to 2,4-dinitrochlorobenzene with viable leukocytes

In a more recent investigation, Harber and Baer (19) found that they were unable to passively transfer allergic eczematous contact sensitivity to 2,4-dinitrochlorobenzene in humans with whole blood transfusions. As an explanation for their findings, they suggest that there may be fundamental immunological differences between tuberculin-type and contact-type eczematous sensitivity.

It is of interest to note that all of the studies so far cited utilized patch testing for the detection of delayed type cutaneous hypersensitivity to simple chemical substances. The only investigation in which intradermal testing was used was that of Haxthausen (20). In his experiments, lymphocyte suspensions prepared from the excised lymph nodes of patients suffering from allergic dermatoses were transferred intradermally to previously normal recipients. When the transfer sites were challenged by patch testing, no positive reactions were found. However, when similarly prepared transfer sites were tested by the intradermal injection of antigen, positive delayed type reactions were obtained in most cases.

The purpose of the present investigation was to determine whether delayed type cutaneous hypersensitivity to intradermal procaine could be passively transferred by viable leukocytes and by leukocyte extracts.

METHODS AND MATERIALS

Separation of Viable Leukocytes: (The method used was a modification of the technic of Minor and Burnett) (21). Bovine fibrinogen (fraction 1, Armour) was used to accelerate the erythrocyte sedimentation rate of sensitive donors. One hundred ml. of whole venous blood was obtained from each donor. The blood was drawn into 50 ml. syringes which had been previously rinsed with heparin (10 mg. per ml). It was then transferred in 25 ml. quantities to test tubes, each containing 3 ml. of fibrinogen solution (45 mg. per ml.). The tubes were inverted several times to ensure complete mixing. The mixture of blood, heparin and fibrinogen was next transferred in 10 ml. quantities to conical centrifuge tubes which were placed at an angle of 60 degrees to the horizontal in a water bath at 37° C. Sedimentation of erythrocytes began in about five minutes and was completed in approximately thirty minutes. As sedimentation proceeded, the suspension of leukocytes in plasma which was formed above the erythrocytes was gently pipetted away at three minute intervals and transferred to graduated capillary tip centrifuge tubes. The cell suspensions thus obtained were centrifuged at 1500 r.p.m. for fifteen minutes. The supernatant plasma was decanted. The cells were packed in a single capillary tip centrifuge tube and washed twice with normal saline. Following washing and recentrifugation, a layer of erythrocytes was seen to be present at the bottom of the tube below the leukocytes. These were removed by aspiration through an 18 gauge spinal puncture needle. The final yield was 0.30–0.45 ml. of washed packed leukocytes per 100 ml. of venous blood. For injection the entire yield of leukocytes was suspended in 2 ml. of normal saline. Sterile precautions were observed throughout, and all glassware was siliconized.

Preparation of Leukocyte Extracts by Freezing and Thawing: The packed leukocytes obtained from 100 ml. of venous blood were suspended in 2 ml. of normal saline in a test tube which was alternately immersed in a bath of dry ice and isopropyl alcohol at 70° C, and in a water bath at 37° C. The freezing and thawing procedure was repeated ten times. The preparation was then centrifuged at 1500 r.p.m. for fifteen minutes. The supernatant leukocyte extract was decanted and retained for injection.

Preparation of Leukocyte Extracts by Sonic Vibration: The packed leukocytes obtained from 100 ml. of venous blood were suspended in 2 ml. of normal saline in a test tube which was subjected to sonic vibration at 45 kilocycles per second for seven minutes. (Branson AP-10-B generator with T-32 transducer.) The preparation was then centrifuged at 1500 r.p.m. for fifteen minutes. The supernatant leukocyte extract was decanted and retained for injection.

Microscopic Examination: Microscopic examination of the viable leukocyte preparations showed about fifteen per cent contamination with erythrocytes. Seventy-five to eighty per cent of

the leukocytes were seen to be viable on supravital staining with methyl red and Janus green. The freezing and thawing and sonic vibration procedures produced complete structural disintegration of the leukocytes as determined by microscopic inspection.

Technic of Passive Transfer: The suspensions of viable leukocytes or leukocyte extracts were injected intradermally in the upper arm of the recipients. The entire yield from 100 ml. of donor blood was given to each recipient. Because the 2 ml. volume of viable leukocyte suspensions or leukocyte extracts was too great for a single injection, four intradermal injections of 0.5 ml. were given in adjacent sites. There was a minimal amount of local inflammatory reaction which usually subsided in twenty-four hours.

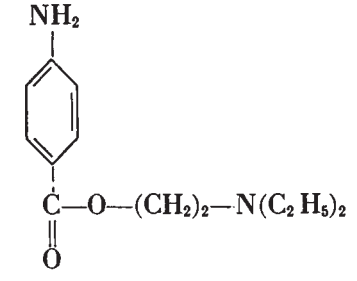
Skin Testing: Skin testing was performed by the intradermal injection of 0.1 ml. of antigen through a 26 gauge hypodermic needle. The test sites were inspected in 6, 24 and 48 hours. The size of the skin test reaction was recorded in millimeters by measuring right angled diameters of induration and erythema at the time of maximum intensity. Any reaction greater than 5 mm. × 5 mm. was considered to constitute a positive test.

EXPERIMENTAL PROCEDURES AND RESULTS

Cellular Transfer of Delayed Cutaneous Hypersensitivity to Procaine with Viable Leukocytes: Eight patients with a history of local allergic edema due to injected procaine, and showing strongly positive delayed type skin reactions to procaine, were selected as leukocyte donors. The recipients were healthy young adults who were shown to have negative intradermal skin tests to procaine prior to the transfer experiments.

Eleven passive transfers of viable leukocytes from procaine sensitive donors to procaine negative recipients were performed. (Three of the procaine sensitive patients were used as donors on two occasions). The recipients were tested with intradermal procaine at the site of injection, and on the volar surface of the opposite forearm at intervals of two, four, seven and sixty days after transfer.

The results of skin testing the recipients with procaine following the transfer of viable cells are summarized in Table 1. It will be seen that six of eleven previously normal recipients developed temporarily positive delayed type skin reactions to procaine. The hypersensitivity of the recipients to procaine was not restricted to the site



Procaine

FIG. 1

TABLE 1

RESULTS OF PASSIVE TRANSFERS WITH VIABLE LEUKOCYTES

Donor	Donor's Skin Test to Procaine (mm.)	Recipient	Recipients' Skin Tests to Procaine (mm.) Day 2	Day 4	Day 7	Day 60
B.A.	10 x 12	F.R.	5 x 5	10 x 10	10 x 10	Neg.
H.A.	30 x 20	R.F.	Neg.	Neg.	Neg.	Neg.
O.R.	45 x 25	F.I.	5 x 5	10 x 10	10 x 10	12 x 10
O.R.	45 x 25	B.O.	7 x 7	5 x 5	-	-
H.O.	35 x 30	G.R.	Neg.	Neg.	Neg.	Neg.
B.R.	15 x 15	H.A.	Neg.	Neg.	Neg.	Neg.
S.T.	40 x 35	P.E.	Neg.	Neg.	Neg.	Neg.
S.T.	40 x 35	B.U.	Neg.	5 x 5	-	-
R.I.	35 x 25	E.D.	Neg.	Neg.	Neg.	Neg.
R.I.	35 x 25	F.A.	Neg.	11 x 8	10 x 10	-
G.U.	50 x 50	K.R.	3 x 3	11 x 6	10 x 9	Neg.

of transfer, but, in each case, was also present in the skin of the opposite arm.

With the exception of donor B.A., the leukocytes obtained from donors showing the largest skin reactions produced the greatest degree of recipient hypersensitivity. The importance of a recipient factor governing the outcome of the transfer is illustrated by the fact that cells taken from donors R.I. and S.T. produced a transfer in one recipient, but not in another. The average duration of the transferred hypersensitivity is difficult to estimate from the data available. Unfortunately, only three of six positive recipients were available for testing at sixty days. Of these, only one was positive at sixty days.

Attempts at the passive transfer of procaine hypersensitivity with plasma were unsuccessful.

Cellular Transfer of Delayed Cutaneous Hypersensitivity to Procaine with Leukocyte Extracts: The passive transfer experiments were repeated using leukocyte extracts prepared by freezing and thawing or by sonic vibration. Five of the eight procaine sensitive patients used in the passive transfer experiments with viable leukocytes again acted as leukocyte donors. (Tables 2 and 3). Three of these five leukocyte donors (O.R., R.I. and G.U.) had produced successful transfers with viable leukocytes.

The results of the cell extract transfers are summarized in Tables 2 and 3. The tables show that, following the intradermal injection of leukocyte extracts obtained from procaine positive patients, two out of six previously normal recipients developed temporarily positive delayed type skin reactions to procaine. As with the viable leukocytes, the transferred cutaneous hypersensi-

FIG. 2 Delayed intradermal reaction (10 mm. × 10 mm.) to procaine in previously normal recipient F.I. ten days following transfer of viable cells from donor O.R.

TABLE 2

RESULTS OF PASSIVE TRANSFERS WITH LEUKOCYTE EXTRACTS PREPARED BY SONIC VIBRATION

Donor	Donor's Skin Test to Procaine (mm.)	Recipient	Recipients' Skin Tests to Procaine (mm.) Day 2	Day 4	Day 7	Day 90
G.U.	50 x 50	L.O.	6 x 10	6 x 10	Neg.	Neg.
R.I.	35 x 25	N.E.	Neg.	Neg.	Neg.	Neg.
H.A.	30 x 20	H.Y.	Neg.	Neg.	Neg.	Neg.

TABLE 3

RESULTS OF PASSIVE TRANSFERS WITH LEUKOCYTE EXTRACTS PREPARED BY FREEZING AND THAWING

Donor	Donor's Skin Test to Procaine (mm.)	Recipient	Recipients' Skin Tests to Procaine (mm.) Day 2	Day 4	Day 7	Day 90
O.R.	45 x 25	G.O.	Neg.	Neg.	Neg.	Neg.
R.I.	35 x 25	T.H.	5 x 8	Neg.	Neg.	Neg.
H.O.	35 x 30	I.N.	Neg.	Neg.	Neg.	Neg.

tivity was also present in the arm opposite to the transfer site. The leukocyte extracts in the two successful transfers were obtained from donors whose viable cells had also produced positive cellular transfers. One of these extracts was prepared by sonic vibration, (G.U.), and one by freezing and thawing (R.I.). The greater degree of transferred sensitivity to procaine was found in the recipient who had received an extract prepared from the leukocytes of the most sensitive donor (G.U.).

CONCLUSIONS

Epstein and Kligman (17) attributed their success in transferring delayed type contact (*i.e.* patch test) allergy to simple chemical substances to the use of large amounts of leukocytes obtained from exquisitely sensitive donors. They also found the chemical configuration of the antigen to be of importance in determining the success of the transfer. In the present investigation, the donors were only moderately sensitive, relatively small volumes of leukocytes were used in the transfers, and procaine is not by reputation an extremely potent sensitizer. Nevertheless, six positive transfers of delayed type hypersensitivity to procaine were obtained in eleven attempts with viable cells. A possible explanation for these findings is that intradermal testing may be a more sensitive method than patch testing for detecting cellularly transferred delayed type hypersensitivity.

Thus, it is of interest that intradermal testing is always employed in the detection of cellularly transferred delayed type hypersensitivity to microbial antigens, whereas patch testing is traditionally utilized in transfer studies with simple chemical substances. If it is assumed that intradermal testing is more sensitive than patch testing, then the consistently successful cellular transfers obtained with microbial antigens, and the highly variable results obtained with simple chemical substances, might be explained as a function of the method of testing for delayed type cutaneous hypersensitivity. The validity of this hypothesis is supported by the experiments of Haxthausen (20) in which it was found that transferred delayed type hypersensitivity to simple chemical substances was detectable by intradermal testing, but not by patch testing. Under the conditions of the present study, the advantages of intradermal testing are clearly demonstrated by the observation that only forty per cent of patients with local allergic edema due to procaine show positive patch tests to procaine in contrast to the one hundred per cent reaction rate with intradermal testing (4). Perhaps intradermal tests, where feasible, should be used more frequently than they are at present in the diagnosis of allergic contact dermatitis.

In cases of delayed type hypersensitivity to procaine, the method by which the antigen is brought into contact with the sensitized tissue greatly influences the nature of the ensuing allergic response. Repeated contact of the epidermis with procaine as occurs, for instance, in sensitized physicians produces an eczematous dermatitis. Repeated subcutaneous injection, on the other hand, results in local allergic tissue edema in susceptible individuals. The phenomenon of local allergic tissue edema would undoubtedly occur with simple chemical substances other than procaine (or chemically related local anesthetic agents) were they administered in the same way as procaine or its analogs. This class of drugs is unique in that they are among the very few simple chemical substances in modern medicine which are frequently and repeatedly injected subcutaneously in the same individual.

An analogous type of reaction occurs with the microbial antigens contained in bacterial vaccines. Patients suffering from bronchial asthma due to bacterial hypersensitivity who are given subcutaneous injections of bacterial vaccine will frequently develop delayed allergic edema of the subscutaneous tissues at the site of injection. This reaction is grossly indistinguishable from allergic tissue edema due to procaine. Furthermore, intradermal testing with the vaccine responsible for the allergic tissue edema will invariably produce a delayed type cutaneous reaction.

A successful transfer of delayed type hypersensitivity with the cell-free supernatant from cells disrupted by sonic vibration has not previously been reported in humans. In guinea-pigs, however, Jeter, Tremaine and Seebohm (22) have reported the transfer of delayed hypersensitivity to 2,4-dinitrochlorobenzene with extracts of leukocytes prepared by sonic vibration. In the present investigation, delayed type hypersensitivity to procaine was transferred in one of three attempts with leukocyte extracts prepared by

sonic vibration. The success of the transfer in this one case may have been due to the sensitivity of intradermal testing, or perhaps to the fact that the cell donor used was the most allergic to procaine in the entire series. It should be remembered that passive cellular transfers of delayed type hypersensitivity have so far been obtained in humans with viable leukocytes (6), with leukocytes disrupted by freezing and thawing (8, 23), with cell-free supernatants separated from cells disrupted by freezing and thawing, (8, 23) and with cell-free blister fluid (24). The so-called cell-free fluids used in cellular transfer studies must contain leukocyte fragments of unknown size which are responsible for the transfer. There is, therefore, no theoretical reason why cell fragments prepared by sonic vibration cannot produce similar results under suitable experimental conditions.

Delayed type hypersensitivity to procaine was also transferred by cell extracts prepared from leukocytes disrupted by freezing and thawing. This finding is in keeping with previously reported results on the transfer of delayed cutaneous hypersensitivity to microbial antigens.

SUMMARY

Delayed tuberculin type intradermal reactions to procaine are found in patients who develop allergic edema of the subcutaneous tissues twelve to twenty-four hours after local infiltration with procaine. The purpose of this study was to find out if delayed cutaneous hypersensitivity to the intradermal injection of procaine could be passively transferred by viable leukocytes, and by leukocyte extracts.

Eight patients with a history of allergic subcutaneous edema due to procaine and strongly positive delayed type intradermal reactions to procaine were selected as donors. Living leukocytes (0.3–0.4 ml.) were isolated from the peripheral blood of each of the donors and injected intradermally in individual procaine negative recipients. When the recipients were tested with intradermal procaine following the transfer, six out of eleven developed positive delayed type skin reactions to procaine.

When the experiments were repeated using cell-free leukocyte extracts prepared by freezing and thawing or by sonic vibration, successful transfers were obtained in two out of six cases.

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